# THE OSTEOGENESIS OF THE BASE OF THE SAURIAN CRANIUM AND A SEARCH FOR THE PARASPHENOID BONE.

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(Forty Text-figures.)

Apparently the first description of a parasphenoid bone on the base of the embryonic skull of a reptile was that of Gaupp in his description of the development of the bony skull of *Lacerta* in Hertwig's "Handbuch" in 1906. Since that date various observers have recorded the presence of the bone on the base of other embryonic lacertilian skulls and on the base of embryonic chelonian and crocodilian skulls.

In every instance the bone in question was said to have a very brief independent existence; very rapidly it fused with the basisphenoid bone.

De Beer tells us that in the process of this fusion with the basisphenoid, the parabasal canal—for the ramus palatinus facialis and the palatine terminal branch of the internal carotid artery—is formed between the bones.

In the birds, W. K. Parker described basitemporal bones on the base of the embryonic skull. These were said to fuse later with the basisphenoid and basioccipital bones. These also have, of later years, been identified as parasphenoidal ossifications and have been described as supplying the floor of the parabasal canal, at least in its posterior portion.

This parasphenoid bone of the sauropsidan embryonic skull differs markedly from that of the fishes and amphibians. Though obvious, the points of difference may be itemized here with advantage: (1) The bone fuses early and completely with the basisphenoid; (2) in the result, its independent individuality is a very brief one; (3) its appearance in point of time coincides with ossifications in cartilage and is later than all the other membrane bones.

In view of these differences it was not possible to regard the identification of this tiny squame of bone as the parasphenoid without suspicion. With a view to investigating the question, material for the study of the ossification of the parasphenoid bone in fishes, amphibians, reptiles, and birds, has been collected during the last ten years and the results of the examination of that material are presented here.

It may be stated at the outset that in only one instance amongst the many sauropsidans studied was a bone which might be regarded as a membrane bone found below the cartilaginous basis cranii. This, as will be seen later, was in the early *Varanus* embryo.

Complete series of developmental stages in which one might study the whole process of ossification of the cranium were only obtained in a few instances, but as these included examples of amphibian, lacertilian, chelonian, and avian development, they provided comparative material which enabled one to interpret the pertinent examples amongst the collection of incomplete series and isolated stages of development of reptiles and birds which have come to hand during these years.

### i. *Fishes and Amphibians.*

These were studied with the object of observing the relation of the ossifying parasphenoid bone to the superjacent cartilaginous basis cranii, and, as the identity of the bone is in no way in doubt, little need be said. Thanks to the kindly assistance of numerous colleagues, whom I have thanked elsewhere for their cooperation, it has been possible to study an extensive range of Urodeles and Caecillians, as well as two very complete anuran series and several isolated stages of other anuran forms. Amongst

the fishes, material has been available for the study of the development of the parasphenoid in a range of modern acanthopterygian crania, and I have also had for study two stages in the development of *Polypterus*, for which I have to thank Professor Graham Kerr.

Throughout the whole of these lower vertebrata the parasphenoid bone was found to ossify at the same time as the rest of the membrane bones. In every instance a very definite perichondral membrane and, in many instances, connective tissue intervened between the cartilage of the basis cranii and the developing parasphenoid bone. In every instance in which a variety of developmental stages permitted one to make the observation, it was observed that the development of the parasphenoid bone had progressed far before there was any change indicating ossification of the cartilage of the basis cranii. Only in the caecillians was fusion of the parasphenoid bone with a basicranial cartilage bone observed. In several of these the parachordal cartilages were seen unchanged and enclosed by their perichondral membrane, with the parasphenoid well developed and extending laterally beyond the cartilages. These parachordal rods continued backward to join the narrow occipital basicranial bar which, itself, was in process of ossification. Fusion between this occipital ossification and the hinder end of the parasphenoid was observed in later stages of development. It is believed that the basisphenoidal region of the base of the skull in the caecillians is ossified only by the parasphenoid. This same condition is also observed in some urodele skulls, but in these the cartilaginous basis cranii is more extensive and persists in adult stages. Fusion between the parasphenoid and the overlying endochondral basicranial and otocranial elements is to be seen in some anuran skulls, but the fusion takes place some time after the metamorphosis has been completed, and is to be compared with the closure of the sutures which takes place between so many of the bones in the skulls of higher vertebrates.

## ii. *Reptiles.*

### A. Lacertilia.

1. *Physignathus lesueurii* (Figs. 1–8).

The eggs of this agamid lizard take one hundred and eleven to one hundred and seventeen days to hatch out. I have been fortunate in obtaining several hundred of the eggs at all stages of incubation, and have been able to study the whole process of ossification of the skull. The head length changes very slightly during the last five weeks of development. At the beginning of this last five weeks the complete chondro-cranium shows no sign of ossific changes, but several of the membrane bones have commenced ossifying. Two weeks later all the membrane bones are well established and early ossific changes are apparent in the cartilage of the basis cranii and in the upper anterior portion of the otocrane. There is no bony formation whatsoever below the cranial base in the basisphenoidal or occipital regions. The ossification of the chondrocranium is not completed until three to four weeks after the young hatch out.

The great majority of the eggs were laid during the last week of October and the first week of November, and as twelve to fifteen were laid in the one hole at the one time they were always collected in batches. This made it possible to date every specimen preserved with a high degree of accuracy, by the simple procedure of always leaving the last specimen in every batch to hatch out. Each batch, as collected, was dated provisionally as having been laid on November first, with a probable margin of error of seven days; they were then allowed to hatch for the period required to fill a gap in the series obtained at the time of their collection; all but one would then be preserved on successive days; when the last hatched out, one corrected the error in their estimated age. The numbering of my stages is an indication, with a fair degree of accuracy, of the period of hatching. One hundred and fourteen days have been taken as the full period of incubation.

Magnified images of the sections were obtained with a vertical projector made for me by Messrs. E. Esdaile and Sons of Sydney. All the drawings have been made by tracing these projected images.

Ossification of the membrane bones commences about the seventy-fifth day and by the eighty-fifth all are well established. This eighty-fifth stage is of particular interest. The chondrocranium has reached its full development and shows no ossific changes anywhere. The internal cerebral branch of the internal carotid artery is seen here as it passes mediad, rostrad and dorsad to reach the pituitary fossa, lying embedded in a groove on the cartilaginous basis cranii. This groove is open antero-inferiorly (Figs. 1 and 2).*

Particular interest attaches to the situation of this artery at this stage, because the relation of the vessel to the basis cranii in earlier stages might very easily lead to erroneous interpretations, had one not been able to study this stage. From the thirty-fifth day, and perhaps somewhat earlier, the chondrocranium has developed all its parts, and from then on undergoes little change. Thereafter, however, the basis cranii gradually undergoes considerable dorsi-ventral thickening, and this reaches its maximum by the eighty-fifth day. Before this thickening takes place the cerebral artery reaches the pituitary fossa without being in contact with the cartilage at all. Figure 3 is taken from a specimen at stage thirty-five, and represents the same region as the illustrations of stage eighty-five. The cranial basal cartilage is actually thinner than the drawing would lead one to believe because the section is slightly inclined to the transverse vertical plane. The head length of stage eighty-five was 10 mm.

In stage ninety, head length 10·8 mm., the only change of note is that the flange of cartilage which stands down and slightly forward behind the cerebral artery has undergone early ossific change.

In stage ninety-two, with a head length of 11·0 mm., spicules of bone are visible in this flange; and the upper surface of the strong membrane which closes the pituitary fossa below has become invaded by osteogenetic tissue. This tissue is continuous with that present along the edge of the cartilage behind the artery.

Apparently it is this ectochondral ossification of the basisphenoidal cartilage which has been identified as the postero-lateral wings of the parasphenoid.

In stage one hundred and five, with a head length of 12·0 mm., the median piece of bone which has been regarded by authors as parasphenoidal is present above the subpituitary membrane, forming a complete bony bar across the floor of the fossa (Fig. 4). In the next section behind this, however, this bone is found to be in complete continuity with lamellae which almost completely surround the trabecular cartilages and which are, beyond doubt, ectochondral ossifications of the cartilage which, itself, may be seen undergoing early ossific changes on each side (Fig. 5). In the section next behind this the whole of both cartilages are found to have broken down and to be entirely surrounded by bony lamellae of ectochondral origin; furthermore, there is intramembranous extension from this latter bone dorso-laterally, most extensive on the left side (Fig. 6). In the next section the union of the pericartilaginous bone lamella of the left side with the ventral table of the basipterygoid ossification is found (Fig. 7). The palatine artery and ramus palatinus facialis, lying on the upper surface of the last lamella, are clearly not resting on a parasphenoidal ossification. Behind the division of the vessel, the internal carotid artery and the ramus palatinus facialis are

* The lettering on the Text-figures is as follows: *A.c.e.*, arteria corotis externa; *A.c.i.*, arteria corotis interna; *Ang.*, angular bone; *Ant.s.can.*, anterior semicircular canal; *Art.c.* and *Art.cer.*, arteria cerebralis; *Art.pal.*, arteria palatina; *Art. vert.*, arteria vertebralis; *B.sph.* and *Ba.sph.*, basisphenoid bone; *Can.p.b.*, canalis parabasalis; *Dent.*, dentary bone; *D.m.*, dura mater; *Epi.pt.*, ascending process of the quadrate; *Eust.r.*, eustachian canal; *Men.pt.*, meniscus pterygoideus; *Mk.*, Meckel's cartilage; *M.pg.*, pterygoid muscle; *M.rect.ext.* and *Mus.rect.ext.*, external rectus muscle; *N.ph.*, eustachian aditus; *O.c.b.*, original boundary of the cartilage; *Opt.ch.*, optic chiasma; *Os.pal.*, palatine bone; *Os.pg.*, pterygoid bone; *P.ant.* and *Pila ant.*, pila antotica; *P.ch.*, proliferating perichondrium; *P.c.o.*, perichondrium cut obliquely; *Ph.*, pharynx; *Pit.*, pituitary body; *Pit.ant.*, pituitary body, anterior lobe; *Pit.post.*, pituitary body, posterior lobe; *Pit.v.*, pituitary vein; *P.sph.* and *Pr.sph.*, presphenoid ossification; *Pr.b.pt.*, processus basipterygoideus; *Pro.ot.*, prootic cartilage or bone; *Pr.pal.pt.*, palato-pterygoid process; *Pt.str.*, subpituitary membrane; *Qu.*, quadrate; *R.sph.* and *Ros.sph.*, Rostrum basisphenoidei; *T.m.*, Taenia marginata; *Trab.*, trabecula; *Tr.cr.*, trabecular crest; *Ty.*, tympanic cavity; *Ven.*, a vein or a venous sinus; *Vena c.l.*, vena capitis lateralis; *Ven.orb.* vena orbitalis; *Ven.sin.*, a venous sinus.

found lying in a canal which is definitely surrounded entirely by basisphenoid bone (Fig. 8). Where these two structures enter that canal posteriorly they lie in a groove on the side of the basisphenoid with only the ventral table of that bone below them.

There are two aspects of the findings in these embryos which lead one to feel confident that no parasphenoid bone is developed. Firstly, the appearances certainly justify the belief that the only squames which might be identified as parasphenoidal are in reality only intramembranous extension of ectochondral ossifications, basisphenoidal in origin. If, one the other hand, we were to regard these intramembranous extensions as entirely membranous in origin, it would be difficult to understand their extremely brief independent existence. In estimating the age of the stages, a possible error of four days is admitted. Assuming that my stage ninety-two would have taken one hundred and seventeen days to hatch out, then it should have been numbered
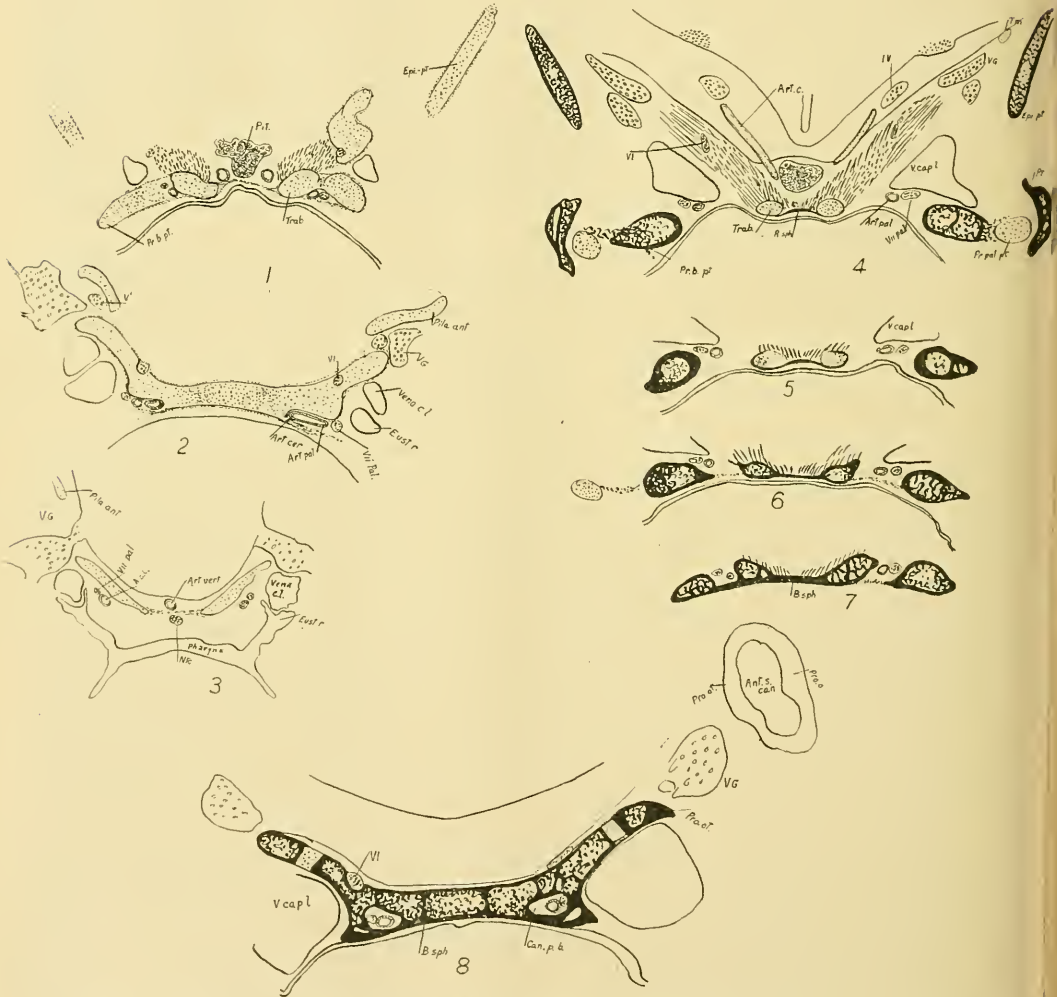


Fig. 1.—*Physignathus.* Stage 85. Transverse section through the pituitary fontanelle just behind the root of the basipterygoid process.

Fig. 2.—Another section through the same specimen, just in front of the junction of the pila antotica to the trabecular crest.

Fig. 3.—*Physignathus.* Stage 35. This section is from approximately the same location as that illustrated in Fig. 2.

Figs. 4-8.—*Physignathus.* Stage 105. Transverse sections through the pituitary and post-pituitary region.

cartilage in process of ossification.     The pila antotica and anterolateral corner of
the basisphenoid cartilage are seen on either side of the pituitary gland and extending
dorsolaterally thereto.     The posterior end of the external rectus muscle attached to
the perichondrium of the trabeculae lies beneath the two internal cerebral arteries and
the pituitary gland, the abducent nerve lies amongst the muscle fibres beside the artery.
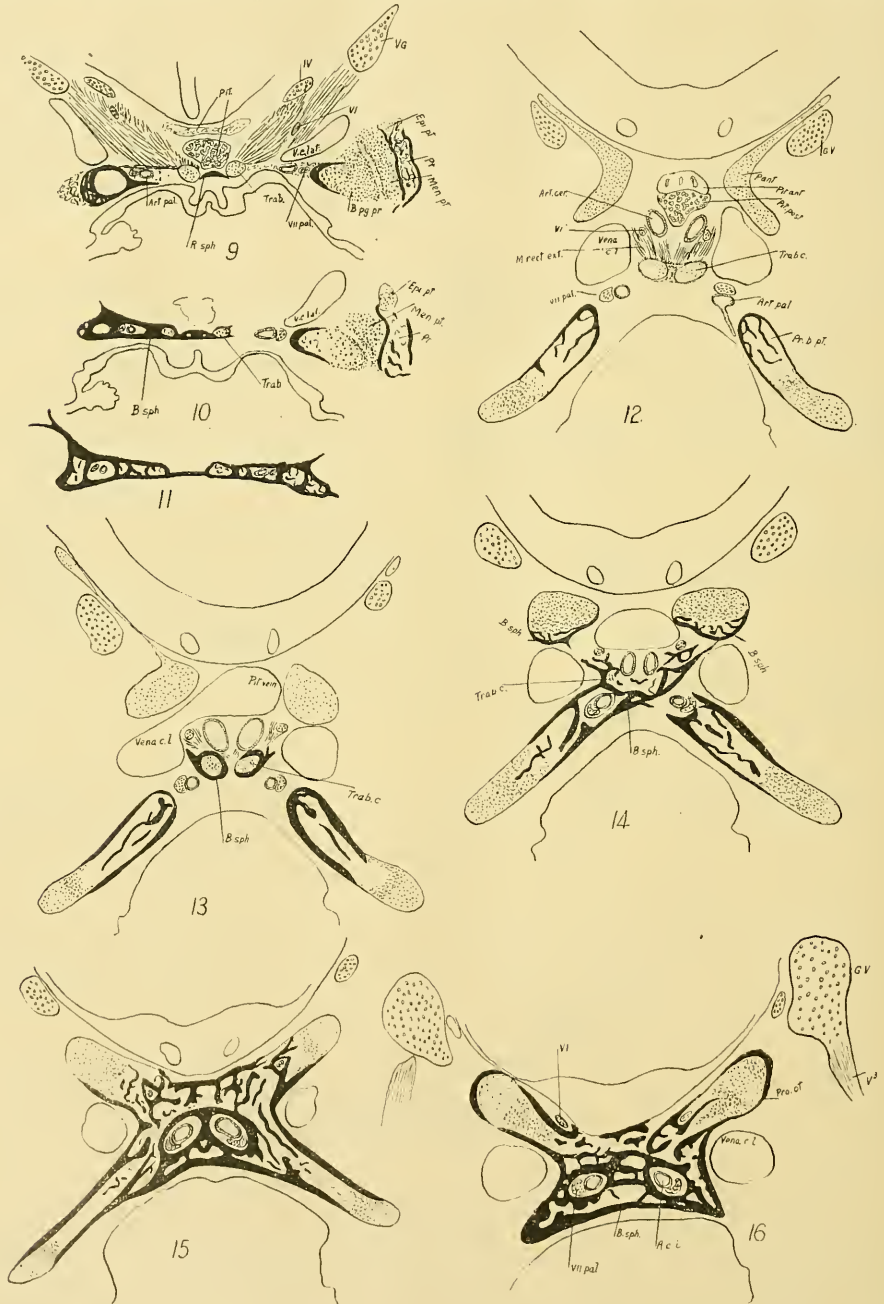


Figs. 9-11.—*Diphorophorus*.     Stage B.     Transverse sections through the pituitary region.
     Figs. 12-16.—*Tiliqua*.     Stage B.     Transverse sections through the pituitary and post-
pituitary region.

The resemblance of these last features to those described for *Lygosoma* by Pearson will be noted. The internal carotid artery has, of course, been cut in front of its division; the palatine branch, accompanied by the ramus palatinus facialis is situated, as will be seen from later sections, in front of the middle of the height of the junction of the basipterygoid process and the body of the basisphenoid. The vena capitis lateralis is placed immediately above the last two mentioned structures.

Figure 13 is taken from the next section but one, No. 169. Here one need only remark upon the fact that both the cartilaginous rods are completely surrounded by bone and that the cartilages themselves are undergoing ossific changes.

In the next section, No. 170 (Fig. 14), the complete continuity of the bone, developed ectochondrally, around the posterior end of the cartilage with the upper and lower bony tables of the basisphenoid bone is very evident on the left side. It is equally evident here that the parabasal canal lies enclosed only by the basiphenoid bone.

The two illustrations (Figs. 15, 16) from sections 173 and 175, are produced to show quite conclusively that the parabasal canal lies entirely within the basisphenoid bone. These sections provide definite evidence that no parasphenoidal ossification is present on the base of the skull.

4. *Varanus* (Figs. 17–21).

Stage A.—Unfortunately my two embryos of this lizard cannot be usefully described by a reference to the head length because the younger is specifically unidentifiable. The stage of development of this embryo is closely comparable with stage 85 in the *Physignathus* series. The membrane bones are all well established, whilst endochondral
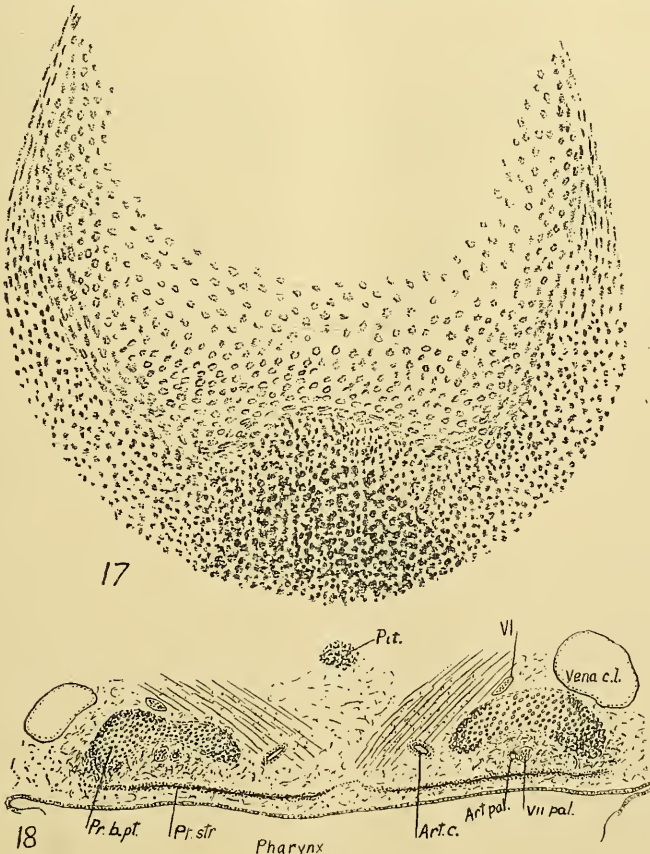


Fig. 17.—*Varanus*. Stage A. A transverse section through the ventral portion of the posterior end of the interorbital septum.

Fig. 18.—*Varanus*. Stage A. A transverse section through the pituitary region.

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ossification is just at its inception, as evidenced by degeneration of the cartilage cells in some places.

Figure 17 illustrates the early changes in the perichondrium on the lower surface of the intorbital septum a short distance in front of the pituitary fontanelle. This early change takes the form of a proliferation of the deep layer of the perichondrium and a breakdown of the membranous outer layer, so that there is no defined boundary between the osteogenetic and the contiguous connective tissues. The process closely resembles that seen in the birds, but the extent of the invasion of the connective tissue is far less. Since the process commences at, or near, the hinder end of the intorbital septum and extends forward as development progresses, a very similar picture is presented at the fore end of the septum in the later stage. The illustration has been drawn from the earlier stage because the wider base in this location offers a bigger field to illustrate.

Figure 18 is taken from the same series of sections as Figure 17; it is through the pituitary region, in front of the arterial canal and just at the root of the basipterygoid process. Here we see the only structure which I was inclined to regard as a parasphenoid bone in the whole of my collection of saurian embryos. It is a thin sheet of densely packed, deeply staining cells which lies beneath the pituitary fontanelle, and which, at its lateral edges, contains tiny spicules of bone (pt.str.). As seen in its entirety from below, this sheet would have the general outline of two right-angled triangles, with their lateral sides about one-half the length of the anterior side, joined at the antero-medial corner and with the postero-lateral corners produced into a short parallel-sided spur. The anterior, sinuated, nearly transverse margin of this sheet of cells lies free with connective tissues both above and below it, and is situated below the hinder end of the pituitary fontanelle. The lateral and the postero-medial margins blend imperceptibly and completely with the perichondrium of the basis cranii.

This sheet is, then, proliferated from the basal perichondrium, and it is only the very wide character of the fontanelle which makes this look different from the conditions found in all the other lacertilian embryos examined.

It may be recorded that the early closure of the basicranial fontanelle is effected in this lizard in precisely the same manner as the pituitary fontanelle, that is to say, by a delicate bony squame developed in cellular tissue which is continuous with the perichondrium and is therefore presumed to have been proliferated from the deep layer of that membrane.

It follows, then, that both of these squames are membrane bones, or that neither of them is.

Stage B.—I have to thank Mr. Kinghorn for four late embryos of *Varanus varius*. These were obtained from a rabbit burrow, and were due to have hatched out in another week or two in all probability. They are very probably about three to four weeks older than the earlier embryos, as judged by comparison with the rate of bone development in *Physignathus*. They are a little further advanced than stage 105 of that species, but not so far as 114.

All the membrane bones are well established and ossification of the chondrocranium is well under way.

There is no trace of any bony lamella or spicule beneath the trabecula communis or anterior end of the pituitary fontanelle (Fig. 19). In the transverse section illustrated, No. 145 of my series C, it will be seen that, though the trabecular cartilages are in process of breaking down prior to ossification, there is no lamella beneath the pituitary vacuity. The dorsolateral ends of both the cartilages are surrounded by ectochondral bone, and this on the left side shows a linear intramembranous extension towards the distal end of the processus basipterygoideus. In the next section, No. 146 (Fig. 20), the continuity of this linear extension with the upper lamella of the basipterygoid ossification proves conclusively that it cannot be regarded as a parasphenoidal ossification, although it is very definitely related to the under surface of the trabecular cartilage. Since there is no bony lamella beneath it, it would appear certain that there cannot be any parasphenoid bone present.

In section No. 149 (Fig. 21) a lamella is found connecting the chondral ossifications of each side.    This has the appearance of the parasphenoid on the base of the caecillian skull, but there is little room for doubt that it is in reality an intra-membranous extension of basisphenoidal ossification.    It is similar in all respects to the lamella which, further forward, connects the upper surface of the ossification of the basipterygoid process to the under surface of the trabecular ectochondral ossification.
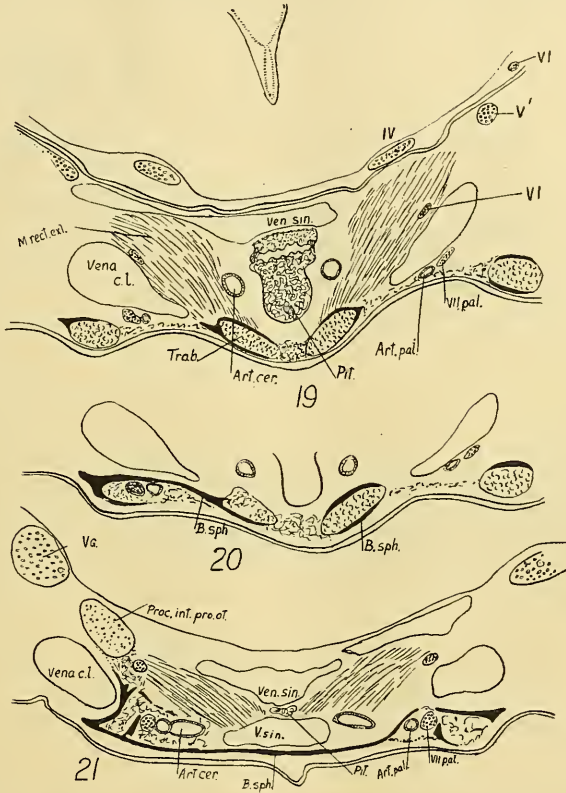


Figs. 19-21.—*Varanus*.    Stage B.    Transverse sections through the pituitary region.

5. *Lygosoma* (*Hinulia*) *guichenotii* (Fig. 22).

The eggs of this common little "Black Fence Lizard" are laid from early in November till the middle of December, and hatch out in about sixty days.

My very complete series of embryos of the species enables me to record that Pearson's (1921) failure to mention the parasphenoid bone was not an omission; there is never any trace of the bone.  De Beer's interpretation of Pearson's report is, therefore, erroneous in the statement that the palatine nerve emerges from the Vidian canal "dorsally to the parasphenoid" (1937, p. 237).

My illustration is drawn from section 59 of one of my series of stage 50, about ten days before hatching.    During the last few days the head length is practically unchanged.

The ossification of both dorsal and ventral surfaces of the basitrabecular cartilages is obvious, and the endochondral ossification is well advanced.    The parabasal canal is clearly lodged in the cartilage on the right side; on the left, slightly further forward, the canal has almost reached its anterior opening.

### Résumé.

It appears then that, except for a tiny squame of bone of fleeting independence, there is no evidence of any parasphenoid bone on the base of any one of the lacertilian embryos studied. It is believed that the squame of bone mentioned is developed from osteogenetic tissue proliferated from the deep layer of the perichondrium of the cranium.

It may be remarked, in conclusion, that the origin of the rectus externus muscle from the floor and side wall of the pituitary fossa, first recorded by Pearson (1921), is not peculiar to *Lygosoma*, but is present in every one of these lacertilian embryos.
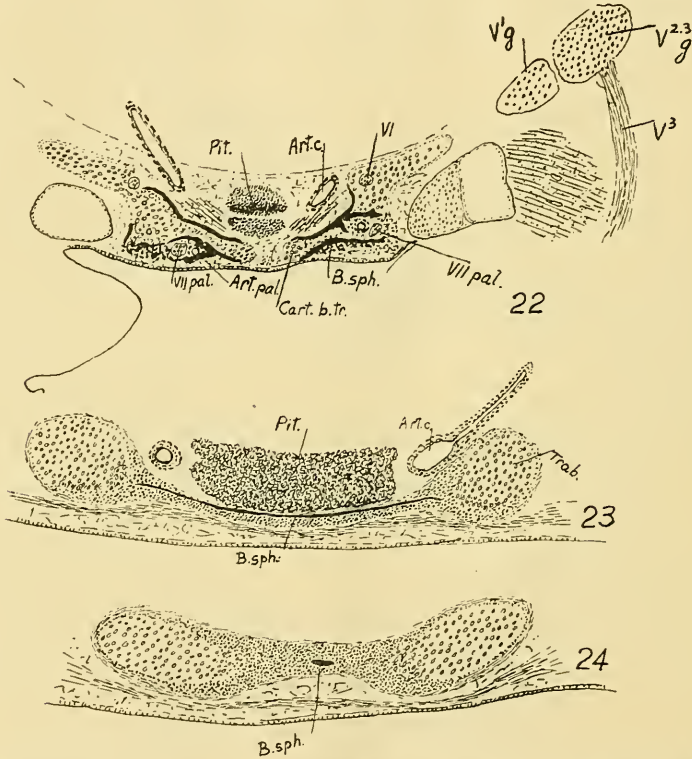
Fig. 22.—*Lygosoma* (*Hinulia*).  A transverse section through the pituitary region.
Figs. 23, 24.—*Chelodina*.  Stage A.  A transverse section through the pituitary region.

### B. Chelonia.

1. *Chelodina longicollis* (Figs. 23, 24, 25).

This "River Turtle" is fairly common in the rivers and lakes of New South Wales, and I have been fortunate in obtaining a number of embryos of different ages.  For these my thanks are due to Mr. Stanley Mabury, the Manager of my farm on the Myall River, N.S.W.

My first two illustrations are drawn from transverse sections through the pituitary and prepituitary regions of an embryo with head length of 9·5 mm.

The appearance here is very similar to that observed in the younger embryo of *Varanus*, but, as it was studied after that, the squame of bone below the pituitary fontanelle was not mistaken for a parasphenoid, as was that other when first seen.

This squame of bone lies, very definitely, above a layer of closely-packed cellular tissue, which is continuous with cellular tissue which lies in contact with the medial surface of the trabecular cartilage, and which is covered both superiorly and inferiorly by the outer, membranous layer of the perichondrium (Fig. 23).  In a section a little further forward the extreme tip of this bony lamella is seen lying in the midst of a

strand of cellular tissue which extends from one cartilage to the other (Fig. 24). There is little reason to doubt that this bony lamella is a membranous extension from endo-perichondral ossification.

Figure 25 is drawn from a section through the head of an embryo with a head length of 9·8 mm. Here, it will be observed, the trabecular cartilages are surrounded by bone, proving quite conclusively that the ossification of the cranium in this region is, at first, endoperichondral. The bone surrounding the cartilages is absolutely continuous with that between the cartilages, and with that which extends dorso-laterally from them. There can be no doubt that we view, here, the basisphenoid bone. It seems, in view of the very complete, and very rapid, fusion of the lamella seen in the younger embryo with the rest of the basisphenoid bone, that it would be wrong to regard the lamella as a parasphenoid bone.

There is no other structure present which could be so identified. There is little doubt, however, that this is the "small plate of bone under the hypophysial fenestra" (de Beer, 1937, p. 259) which has been so identified in other chelonian embryos.

## C. Ophidia.

1. Python.

I have but a single stage in the development of this snake. The chondrocranium is fully formed and most of the membrane bones have commenced to ossify.

Ossification of the basisphenoid is definitely endochondral. Small areas of broken-down cartilage and very early bone formation are present on either side of the pituitary fontanelle. No trace of, and nothing which could be mistaken for, a parasphenoidal ossification is visible. The pituitary fontanelle is supplied with a floor of dense membrane as in the other reptiles studied.

It is generally agreed that there is no parasphenoid developed in the ophidian embryonic skull; this is probably because, in the ophidia, the ossification of the basi-sphenoid is endochondral and therefore the ventral table of the bone is not completed or, indeed, even initiated before the cancellous tissue.

There is no doubt that the general agreement upon the absence of the parasphenoid from the ophidian embryonic skull is correct; there is no such bone here. This fact is to be regarded as evidence that the so-called parasphenoid bone of other reptilian embryonic skulls is in reality only the ventral table of the ectochondrally developed basisphenoid bone, for it is difficult to understand why, if the bone be present in those, it should not be present also in the ophidians.

2 and 3. *Pseudechis* and *Dendrophis*.

These two snake embryos were both taken from the egg just before hatching, and ossification is nearly complete in both of them. The *Pseudechis* is slightly the younger and in it the ventral table of the parasphenoid is complete. The dorsal table shows some areas not yet ossified. No trace of a parasphenoid ossification is to be seen in either skull.

### iii. *Aves.*
### General statement.

The kindly assistance of several friends has made it possible for me to examine the embryos of sixteen different birds in the present investigation. I have to thank Mr. H. C. Mawhiney of St. George, Queensland, for the series of Emu embryos, I am indebted to the late Dr. J. Allan Thomson, late Director of the Dominion Museum, Wellington, New Zealand, for the *Apteryx*, and all but two of the Australian birds were collected for me by Messrs. H. A. Blakeney and J. S. P. Ramsay, who were good enough to make special excursions for them.

It may be stressed that the process of ossification of the avian basis cranii, as described below, is not peculiar to that part of the chondrocranium, but is similar to the process of ossification of the chondrocranium in all its parts, and is characteristic of, and peculiar to, the aves. It is probably causally related to the pneumatization of the bones.

The earliest observed change leading to ossification takes the form of a cellular proliferation of the perichondrium, and is seen first below the trabecular communis and

the trabeculae on either side of the pituitary fontanelle.    This thickening of the perichondrium is particularly marked towards the posterior end of the interbital septum. At the anterior boundary of the pituitary fossa the area of proliferation splits into two, and each half is continued backwards along the ventro-medial surface of the cartilage on each side of the fontanelle, and then on to reach the basicranial cartilages on each side of the basicranial fontanelle.    At the point of divergence of the trabeculae, the area of proliferation not only divides onto the ventra of the two rods, but also extends directly backwards between the diverging rods so that for quite an appreciable distance the areas of proliferation on the ventro-medial surfaces of the cartilages are connected by this proliferation into the tissues below the fontanelle.

Following the cellular proliferation of the deep layer of the perichondrium there is a loss of definition of the outer layer, and this is followed by invasion of the connective tissue in immediate proximity to it by further proliferation of the earlier layer.    This later phase, however, leads to the development of an area, rich in cellular elements, but differing from the early stage in that they are not so closely packed and that the cytoplasm of the majority of the cells takes the basic stains more intensely, and the nuclei do not, therefore, stand out so clearly.    The process continues by further invasion of the surrounding tissue and by the aggregation of the cells into irregularly disposed masses.    Definite osteoblasts next become recognizable, and their appearance is rapidly followed by the formation of bony spicules.    These spicules appear first, for the most part, in contact with the surface of the cartilage, but always in the midst of an aggregation of the cells.    Meanwhile the superficial cartilage cells have undergone early degenerative changes.

The ossification of the base of the interbital septum commences near the posterior end and takes several days to reach the anterior end, so that the whole of these changes in the periosteum leading to early ossification may be studied in a single specimen of appropriate age.    The fourteen and one-half day chick is a perfectly satisfactory example. I have been able to observe the process in *Apteryx,* Emu, *Phalacrocorax, Recurvirostra, Podiceps, Botaurus, Melopsittacus, Acrocephalus, Himantopus, Erythrogonys, Iredipara, Gallus* and *Anas.*

A detailed description of the histological changes at the various stages in this process of ossification will be given elsewhere; here we are interested in the question of the presence or absence of parasphenoidal ossifications on the avian cranial base, and proceed to selected descriptions of observed conditions which throw light on this particular question.

Since the first bony formation is, throughout the birds, always the ventral edge of the interbital septum and the ventral table of the basisphenoidal, otocranial and basioccipital regions, there is always formed a bony squame, triangular in outline and with an elongated splint-like rostral projection.    This is the structure which has been identified as the parasphenoid bone.    Apparently the basisphenoidal wings of this squame may, at times, be separately formed, giving rise to the so-called basitemporals, but none of my many specimens show this feature.

The earliest portions of this "parasphenoid" to be formed are the basiseptal and basisphenoidal, but, quite uninterrupted continued growth carries it back and laterally to underlie the whole of the otocranial and basioccipital regions, as just stated. Therefore, if there be a parasphenoid bone here at all it should be credited with covering the whole of the basis cranii, and not merely part of it; any limitation must be by purely arbitrary definition.    As against this view, however, there is the fact that, even before the ventral table has covered the areas said to be protected by a parasphenoid, the earliest formed portion of the squame has been added to by endochondral ossification, so that this so-called parasphenoid has already completely fused with the basisphenoid before it has developed to its full superficial extent.    Quite apart from the histological evidence that the whole ventral table is an endo-perichondral ossification, it would surely not be correct to identify as separate entities portions of bone which have so brief an independent existence.    In support of this view, it may be pointed out that the rigid observance of such a procedure would involve the necessity

of recognizing as independent ossifications quite a number of primary centres of
ossification. For example, in the majority of the birds, if not in all, the basisphenoid
bone is ossified from five endo-perichondral centres: one ventral, two on the ventro-
medial wall of the lateral arterial canal, and two on the dorso-lateral wall, all essentially
similar. If the ventral is a membrane bone, so also are the others, and we must
regard the basisphenoid bone as having been formed by the fusion of five membrane
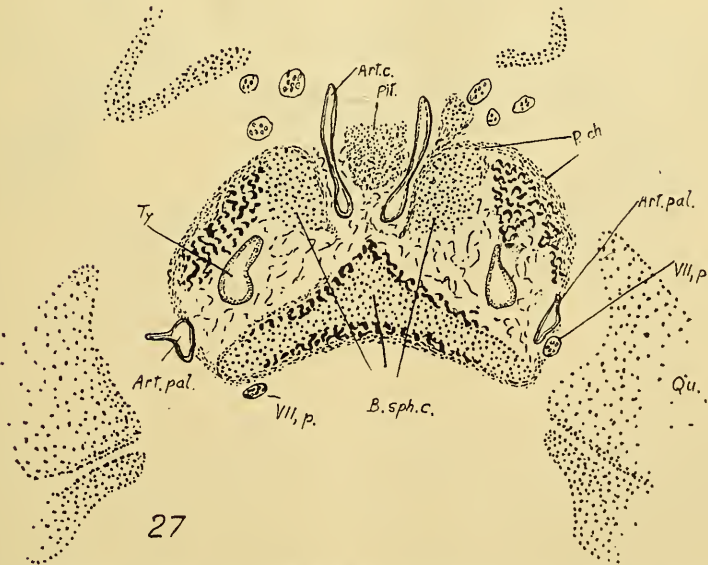bones with the endochondral bone, truly a *reductio ad absurdum*.

There is yet another feature of general occurrence which bears upon the question.
Wherever there is an extension of a chondral bone, the extension is preceded by
invasion of the area by osteogenetic tissue which is proliferated from that which has
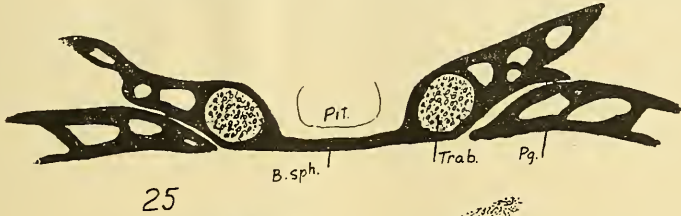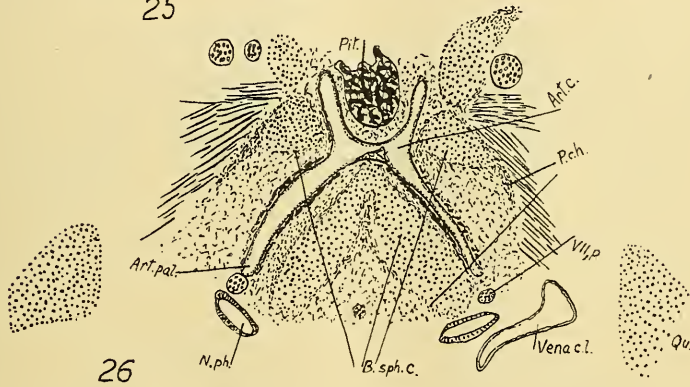


Fig. 25.—*Chelodina*. Stage C. Transverse sections through the pituitary region.
Fig. 26.—Emu, head length 22 mm. A transverse section through the pituitary region.
Fig. 27.—Emu, head length 37 mm. A transverse section from the same location as
Fig. 26.

given rise to the bone from which the extension has taken place. This invading osteogenetic tissue is precisely similar to that which gives rise to the bony squames first formed all round the cartilage.

Finally, further support for the interpretation of the ventral bony squame adopted here is found on comparing the formation of the truly membrano-genetic dentary around Meckel's cartilage with these other squames. Throughout the whole growing period there is no trace of any change in the perichondrium and the osteogenetic tissue is always clearly distinct from that membrane.

### Detailed descriptions.

1. The Emu, *Dromaius novaehollandiae* Latham* (Figs. 26–29).

Stage A.—Head length 22 mm. The section illustrated (Fig. 26) includes nearly the full length of the internal cerebral arteries and their cross commissure, within the lateral arterial canal, and the pituitary fossa. The position is just behind the pituitary fontanelle, and the section shows the trabeculae just after their union at the back of that vacuity. The anterior tip of the notochord lies a little further back.



Fig. 28.—Emu, head length 40 mm. A transverse section from the same location as Fig. 26.

Fig. 29.—Emu, head length 45 mm. A transverse section from the same location as Fig. 26.

Interest attaches particularly to the proliferation of the perichondrium at the mid-line and on either side thereof below the cartilages, from the ventro-lateral corner of the cartilage above the canal and along the dorso-lateral surface of the cartilages below the canal (Fig. 26, P. ch.). Particular interest must attach to the second of these areas of proliferation, for it extends a considerable distance from the cartilage. Later stages will show that the greater part of the loose connective tissue between this band of osteogenetic tissue and the artery will be absorbed to make room for the

* The nomenclature of this communication is taken from a popular work, "What Bird is That?" by Neville Cayley (1939).

anterior recess of the tympanic cavity. The absence of proliferation of the perichondrium around the ventro-lateral corner of the cartilages is noteworthy.

Stage B.—Head length 37 mm. (Fig. 27). The length of the cerebral artery included in the section indicates that this is fairly comparable with the preceding section. Particular interest attaches to the areas of early ossification. It will be observed that they are all in precisely the locations in which proliferation of the perichondrium was observed in the earlier stage.*

The five centres of ossification of the basiphenoid, mentioned above, are seen in this section, but the two on the floor of the arterial canal have fused medially. If there be a true membrane bone shown in the section it must surely be that which lies dorso-laterally to the anterior recess of the tympanic cavity, for it alone is formed independently of cartilage. That is, independently if we disregard the fact that its osteogenetic tissue was proliferated from the perichondrium.

The ventral squame is uninterruptedly continuous with the basi-septal "parasphenoid" rostral splint.

Stage C.—Head length 40 mm. (Fig. 28). The presence of a section of the cerebral artery indicates that the section has been cut in close proximity to the situation of the others. Here the dorsal and ventral tables, below the pituitary fossa, have almost fused, whilst as yet the lateral corners of the cartilage are but little changed.

Stage D.—Head length 45 mm. (Fig. 29). The cartilage below the fossa has been almost completely replaced by bone. It will be observed that this replacement has been brought about by the equal extension of *similar* dorsal and ventral areas of ossification, and that long before this stage was reached both dorsal and ventral superficial squames had lost their identity.

2. *Melopsittacus undulatus* Shaw (Figs. 30, 31).

I have to thank Mr. M. Brennan of Earlwood, Sydney, for a very complete and perfectly preserved series of embryos of this little Parrakeet. He obtained them for me from his own aviary, so that the period of hatching is as accurately known as is possible. The two sections illustrated are both taken from the same transverse series of an embryo of the fourteenth day of hatching.

Figure 30 is taken from a section through the intorbital septum towards its posterior end. This section has been selected for illustration because the presence of a venous sinus between the osteogenetic stromata of the presphenoid and pterygoid bones demonstrates very conclusively that these are not continuous. In other locations they are separated by quite a wide interval of connective tissue or, where they appear to be continuous, by a thin membrane. The question which their apparent continuity in some locations raised was: In view of the fact that pterygoid and palatine bones are, in this bird, developed from one continuous osteogenetic stroma, is that from which the presphenoid basal ossification developed part of that same stroma? Careful study of the sections in front of and behind the sinus, where the apparent continuity is seen, shows that the question may be confidently answered in the negative. It may be remarked that the apparent unity of the two stromata was suggested by their close similarity as well as by the apparent continuity. The illustration is further selected to show the proliferation of the perichondrium below the septum and the massing of the osteoblasts within that proliferation.

The continuity of the stromata of the pterygoid and palatine bones is of interest. It has been observed that it is not unusual in the developing bird head to find the osteogenetic stromata of contiguous membrane bones to be quite continuous in the younger embryos. This may be observed particularly around Meckel's cartilage, where the stromata, from which angular, surangular, splenial and dentary bones are developed, are at first quite continuous. It is of particular interest, in this connection, that the osteogenetic stroma of the articularis, on the other hand, always remains quite definitely separated from those of the contiguous membrane bones.

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* It may be permissible to stress the fact that all the illustrations have been made by direct tracing of the projected image of the section, using a lantern projector especially devised for the purpose; that these tracings were transferred directly for the final drawing and were not copied freehand.

Figure 31a is also from a section through the intorbital septum; this is just a few sections in front of the pituitary fossa. The early invasion of the base of the septum, trabecula communis, by the osteogenetic tissue may be clearly seen here; tiny spicules
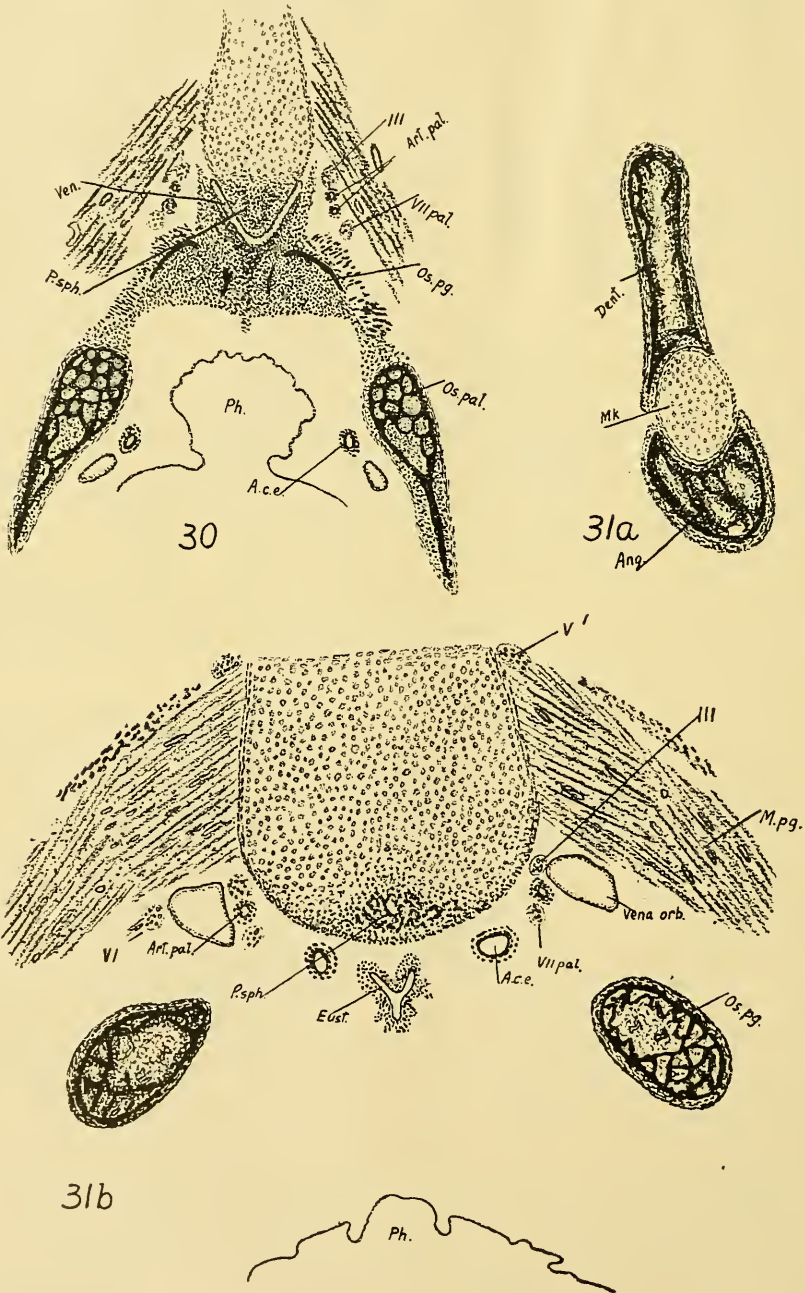


Fig. 30.—*Melopsittacus.* Stage 14 days. A transverse section through the posterior orbital region.

Fig. 31a.—*Melopsittacus.* Stage 14 days. A transverse section through the pre-pituitary region. Fig. 31b.—Transverse section of Meckel's cartilage and related bones, from the same section.

of bone have already been formed. The advanced development of the pterygoid bone may be contrasted with that of the presphenoid ossification.

Figure 31*b* is taken from Meckel's cartilage and two of its investing bones, and illustrates another feature in the same section as Figure 31*a*.

The clear-cut, unchanged surface of the cartilage in contact with the stromata of the investing bones is in strong contrast with the lower end of the intorbital septum, whereon the endochondral presphenoid is commencing to be formed.

3. The Cormorant, *Phalacrocorax varius* Gmelin (Figs. 32–35).

Stage A.—Head length 21·3 mm. Figure 32, from a section cut through the posterior end of the intorbital septum, shows the proliferation of the perichondrium on both sides of the ventral edge of the cartilage, and the absence of the perichondrium below the cartilage between those areas. There is a general similarity between this ectochondral ossification and that of the two pterygoid bones; actually, however, the osteogenetic membrane around the latter two bones is thicker and its cells, besides being more densely packed, stain more intensely.

Figure 33 is from a section cut through the pituitary fossa in front of the arterial canal. Of interest here, is the continuity between the osteogenetic tissue below the ventral table and the osteogenetic tissue pervading the connective tissue between the cartilages and invading the cartilages themselves. The approximate boundary of the
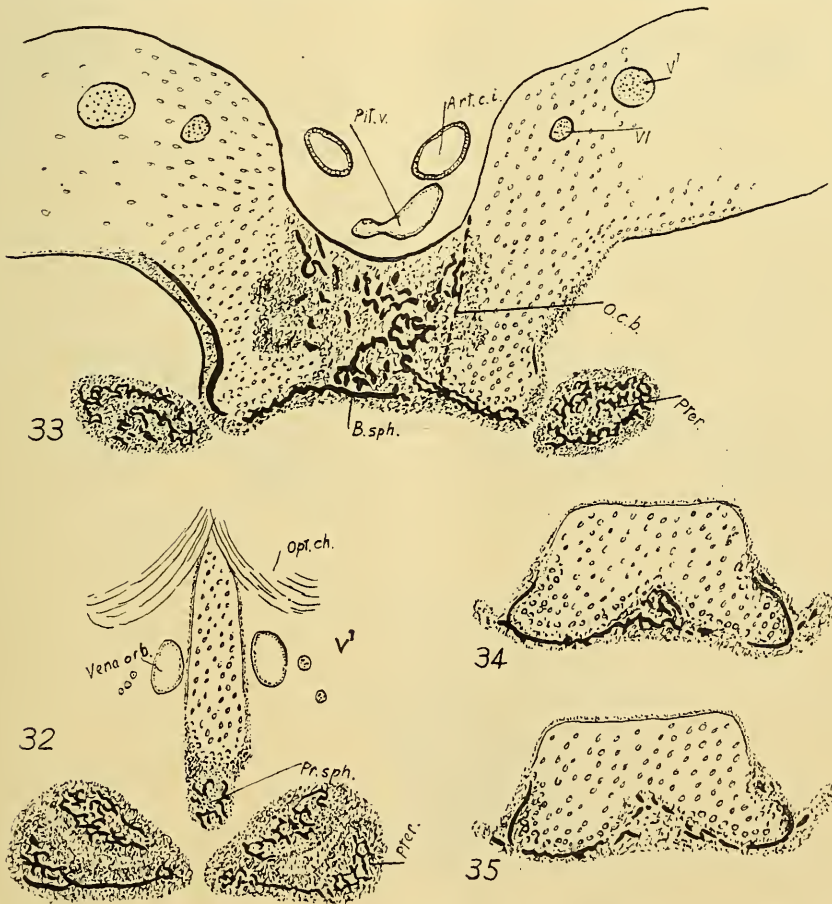


Fig. 32.—*Phalacrocorax*, head length 21·3 mm. A transverse section through the pre-pituitary region.

Figs. 33-35.—*Phalacrocorax*. Transverse section through the pituitary and post-pituitary regions.

cartilage on the right-hand side, before the invasion, is indicated by the dotted line
*O.c.b.* Other features of interest are the presence of the long bony squame on the
ventrolateral surface on the right-hand side, the proliferation of the perichondrium in
this area on both sides, and the very definite line of demarcation between the osteo-
genetic tissue of the true membrane bone, the pterygoid, which makes contact with
the cartilage at this point, and that of the basisphenoid.

Figures 34 and 35 show the continuity between the ventral and lateral squames on
the surface of the cartilage a little further back.

4. The Chick, *Gallus domesticus* Linné (Figs. 36–40).

Stage, 14½ days' incubation at 38°C.—The sections illustrated are numbers 180 to
176 in my own series of this stage; they provide almost conclusive evidence that there



Figs. 36–40.—*Gallus.* Stage, 14½ days. Five consecutive transverse sections through the
pituitary region. Fig. 36 is the hindmost, Fig. 40 the foremost.

is no parasphenoid on the base of this chondrocranium. In the first (Fig. 36) we have the two, so-called, basitemporal bones. These are little squames of bone situated beneath, but at quite a distance from the trabecular cartilages. These sections are each 100μ thick, and in the next (Fig. 37) the cartilage is found to have been not so far distant, at least on the right side, for the section has been cut just behind an abrupt rise dorsad of the postero-ventral surface of the cartilages. In the section next in front of this (Fig. 38) we find complete continuity of the osteogenetic tissue from which the "basi-temporals" are being developed and almost complete continuity between the bones themselves. In the next section (Fig. 39) the bones are joined together in the mid-line and are also continuous with bone which has been deposited on the lateral surfaces of the cartilages and with that which, lying between the cartilages, is invading the one on the right-hand side. In the last section (Fig. 40), as in the fourth, it is quite impossible to define any boundary between two bony elements of different origin.

5. *Apteryx*.

The specimen studied had a head length of 5·5 cm. from tip of snout to the occiput. There was no record as to how far advanced in hatching the chick was, but, since the feathers were but poorly developed, it was probably several days short of the full period. The specimen was one of the collection made by Jeffery Parker many years ago, when he was studying the development of the bird. Notwithstanding its age the preservation was almost perfect.

It was stained in bulk and embedded in nitrocellulose. It was then sectioned to the mid-line in the sagittal plane and then remounted and the remaining half cut trans-versely. A model of the portion we are interested in was reconstructed from these transverse sections.

For this model the drawings were made directly on white blotting paper. After they were cut out, the portions of cartilage undergoing ossification, already indicated on the drawing, were coloured red, the nerves and the Gasserian ganglion were coloured blue, and the cerebral artery dark purple. The drawings were then dipped into paraffin and placed in position before the paraffin cooled.

It was found that the basisphenoid bone had apparently commenced ossification from two centres, ectochondrally and ventrally. This appeared to explain the fact that the basisphenoid region of the chondrocranium was found to be permeated by endochondral ossification throughout its thickness on each side of and slightly behind the pituitary fossa. The upper limit of this ossification was found to fall away ventrad, posteriorly and anteriorly. The whole of the floor of the fossa was ossified, as also was the lateral wall in front of the arterial foramen. This ossification of the cartilage permeated, not only the vertical, but also the lateral thickness of it in this region, and extended out along the remarkably massive basipterygoid boss so that only the core and extreme tip of this latter remained as cartilage. Where the cerebral artery enters its canal it lies between cartilage in front and bone behind, but at its point of emergence into the skull it has bone in front and cartilage behind it. This cartilage behind the artery is continuous with the core of the basipterygoid process. At the periphery of this area of ossification there was found only the thin ventral table of the bone. That this was the ventral table, and not a separate basi-temporal ossification, appeared quite definite from the fact that it was quite continuous with the ventral table below the area of more massive ossification; moreover, this lamellar extension of the bone was definitely intra-perichondral at its edge.

No trace of any extra-perichondral ossification was found anywhere on the base of this chondrocranium.

The prootic bone was found to have commenced ossification in its upper and anterior region, and this ossification was in all respects similar to that of the basisphenoid.

The basioccipital, not included in the model, was also found to be ossifying in a similar manner.

If, as believed, the basisphenoid bone commenced ossifying ectochondrally from two ventrally-situated centres, then it is possible that, at an earlier stage than that studied, the appearances might simulate separate basitemporal squames. In view of their

early complete fusion with the endochondral ossification and their situation above the perichondrium, it would surely be erroneous to identify them as parasphenoidal ossifications.

Besides the four avian embryos which have been selected for special description and illustration, embryos of the following birds have been carefully examined for the present study. All these so closely resemble one or other of those selected that it was felt their description and/or illustration would be merely duplication: *Fulica atra* Linné, two stages, *Recurvirostra novaehollandiae* Vieillot, three stages, *Podiceps* sp., *Thraskiornis molucca* Cuvier, one stage, *Botaurus poecilloptilus* Wagler, one stage, *Acrocephalus australis* Gould, eight stages, *Himantopus leucocephalus* Gould, two stages, *Erythrogonys cinctus* Gould, three stages, *Podargus* sp., two stages, *Iredipara gallinacea* Temm., two stages, and *Anas*, twelve stages.

I had expected to include studies of the development of the osteocranium of the Crocodile. Arrangements had been made for the collection of the necessary material but unfortunately on both occasions the district selected for the collection was visited by disastrous floods, which made collection impossible.

## Summary.

Very complete series of embryos of two Lacertilia, one Chelonian and three Aves as well as single embryos and/or incomplete series of embryos of twenty other Saurians have been obtained and prepared for microscopical examination during the last ten years.

A careful examination of this mass of material has been carried out, and from it embryos of fourteen Saurian species have been selected for description and illustration.

These reveal that—except for a squame of very fleeting independence, which is present below the pituitary fontanelle of a majority of Lacertilian and Chelonian embryos—there is no structure which can be identified as a parasphenoid bone.

There is strong reason to believe that the tiny squame referred to is an intra-membranous extension of that endo-perichondral ossification which was observed to initiate the ossification of the chondrocranium of all Saurians except the Ophidia.

In those reptiles in which the ossification of the basisphenoid commences with the appearance of this squame, it differs in no way from the ossification of the basioccipital bone, which commences with the appearance of a precisely similar squame below the basicranial fontanelle.

Throughout the birds a triangular endo-perichondral squame develops in contact with the cartilage on the base of the skull and in cellular osteogenetic tissue derived, it is believed, from the perichondrium by proliferation of its deep layer.

This triangular squame very rapidly becomes fused with the endochondral bone which develops in the cartilage above it, so that, still quite small, its first-formed portion has already so fused whilst the peripheral portion of the squame has yet to be developed.

Similar squames to this basal one are developed in other relations to the chondrocranium, and in the case of the basisphenoid there are four such others. Therefore, since they *are* all similar, if any be membrane bones, all must be, and under such an interpretation the basisphenoid bone must be deemed to have been formed by the fusion of five membrane bones and one endochondral bone.

This last suggestion is, of course, a *reductio ad absurdum*, and the truth is that the parasphenoid is developed from five similar endo-perichondral centres of ossification.

## Conclusion.

The tiny squames on the base of the chondrocrania of various Saurians, which have been identified as parasphenoidal ossifications, are in reality endo-perichondral ossifications, and no true parasphenoid bone is found on the base of any Saurian skull.

It follows that, in the Saurians the parabasal canal is enclosed by the basisphenoid bone only; except in the Chelonians, where part of its floor is supplied by the pterygoid bone.

These findings support my belief and contention that the reptilian pterygoid bone should be equated with the lateral wings of the amphibian parasphenoid bone.

This equation was first advanced in 1916 (Kesteven, 1916)* and leads on to the equation of the mammalian pterygoids with the reptilian pterygoids and with the same lateral wings of the amphibian bone, an interpretation which was advanced at the same time and which has been supported by several communications since (Kesteven 1919 to 1931) and which has recently been adopted by de Beer on portion only of the evidence advanced in those communications.

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* A list of references to literature is not given here because all but certain of my own communications are listed in de Beer's very fine list at the end of his book on "The Development of the Vertebrate Skull". My own communications were printed in the *Journal of Anatomy* and in the *Records of the Australian Museum*. Cayley's book, referred to in a previous footnote, was published by Angus & Robertson, Sydney.